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STUDIES ON THE ETIOLOGY OF BLACKWATER FEVER

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Despite numerous investigations on blackwater fever the etiology of this disease is still unknown. One school of investigators believes that quinine and malaria are the factors responsible for the disease. The other school contends that there are two distinct disease conditions, one associated with malaria which they designate as quinine hemoglobinuria; the other specific blackwater fever. The first mentioned condition may be produced in cases of chronic malaria by even minute doses of quinine, while the second has a specific etiology, the nature of which is still unknown.

The mechanism of the production of hemoglobinuria during malaria has been studied extensively by Barratt and Yorke (1909). They found that quinine, hydrochloric acid, and sodium hydroxide will induce the necessary hemolysis, but that during life quinine never reaches a concentration sufficient to cause hemolysis. The anuria they attribute to a mechanical blocking of the kidney tubules by the deposited hemoglobin.

In the course of the last year we have had occasion to study 4 cases of clinical blackwater fever. Certain experiments were made with the hope of obtaining facts with regard to the cause for the appearance of blood in the urine. The purpose of this paper is to report briefly such observations as appear to be of interest.

RELATIONS TO MALARIA AND QUININE

All the cases gave a history of malaria. Malaria parasites were found in the blood of each either before or after the attack of hemoglobinuria. During it no parasites were found. Three

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of the cases had tropical malaria (2 with gametes) and in the fourth the benign tertian parasite was found. Two of them possess special interest because the attacks occurred in the hospital after the injection of relatively small doses of quinine and the development of the condition could be followed from the beginning.

A brief description of these two is given below:

S. H., girl, fourteen years. Admitted January 21, 1921. Only ten months in the country. Had repeated attacks of malaria during six months prior to admission. Quinine treatment irregular. Came to hospital because of fear of blackwater fever. Blood examination showed tropical gametes. On January 22, 1921, quinine injections were started, 0.05 gram, 8.45 a.m.; 0.5 gram, 11 a.m.; 0.5 gram, 6 p.m During the night the patient had an attack of blackwater fever: temperature 38.3°C., pulse 120; body and sclerae jaundiced; urine red. Examination of urine: hemoglobin+; albumin±; bile —; red cells —; epithelial cells and casts +. The next day the urine was clear and the temperature returned to normal. Quinine was later given without the development of hemoglobinuria. The patient is now well.

R. L., girl, sixteen years. Admitted March 15, 1921. Repeated attacks of malaria during past year. Quinine treatment irregular. Had two attacks of hemoglobinuria, the last two weeks before entering the hospital. One week after admission to the hospital she received a dose of 0.4 gram of quinine on two successive days and 0.5 gram on the third day. A few hours after the last injection the patient developed hemoglobinuria. Temperature 38.6°C.; pulse 120; body and sclerae jaundiced; urine deep red. Examination of the urine revealed the presence of hemoglobin, albumin, white and epithelial cells and casts. Red cells were rare. No malarial parasites could be found in the blood at this time, but four weeks later, while still in the hospital, the patient had an attack of malaria and parasites of the tertian variety were found.

The history and findings of these two cases are typical of the rest. The attack was characterized by a rise in temperature with a corresponding increase in pulse rate and dark red urine containing hemoglobin, albumin, and casts, but practically devoid of red cells. As the urine cleared, the temperature fell and the condition of the patient improved.

SECRETION OF QUININE

In one case a series of quantitative tests was made of the secretion into the urine of quinine and albumin during the course of the attack. Each specimen passed was examined for the presence of these substances. The results are shown in the table. Unfortunately one specimen was lost and consequently the total quinine excreted could not be determined.

It is of interest to note that the concentration of quinine and albumin in the urine roughly paralleled each other, though albumin and blood continued to be eliminated after tests for quinine were negative. Bile was never present.

*Analysis of urine of blackwater fever patient**

SAMPLE	AMOUNT	SPECIFIC GRAVITY	ALBUMIN	QUININE PER CUBIC CENTIMETER	TOTAL	HEMOGLOBIN	BILE
	cc.		*per cent*	*mgm.*	*mgm.*		
1	80	1.0107	0.18	0.1	8.0	+	−
2	(?)	(?)		(?)	(?)	+	−
3	150	1.010	0.36	0.5	75.0	+	−
4	420	1.009	0.24	0.03	12.6	+	−
5	333	1.008	0.12	0.02	6.6	+	−
6	400	1.008	0.12	0.00	0.0	+	−
					102.0		

* The total quinine injected just before the attack was 500 mgm.

FRAGILITY OF RED CELLS IN BLACKWATER FEVER

A number of observers have attributed the destruction of red cells in blackwater fever to alterations in surface tension. Tests were therefore made to determine the fragility of the cells to hypotonic solutions shortly after the attack of blackwater fever. One drop of defibrinated blood was added to each of a series of tubes containing 1 cc. of salt solution of graded hypotonicity. No difference was observed between the behavior of a normal blood and of that from the blackwater fever patients. In both instances marked hemolysis (about 60 to 70 per cent) occurred after fifteen minutes in a salt concentration of 0.27 per cent, and practically none in one of 0.36 per cent.

EFFECT OF QUININE ON BLOOD CELLS FROM MALARIA AND BLACKWATER FEVER PATIENTS AND FROM NORMAL INDIVIDUALS

These experiments were a repetition of those made by Barratt and Yorke and confirmed their findings. Quinine had the same effect on normal and malarial blood. It required a concentration of 0.2 per cent of quinine hydrochloride to cause laking of each. In one series of tests only was the blood of a blackwater fever patient laked by a somewhat lower concentration (0.15 per cent) of quinine. But to attain even this concentration in the body, it would be necessary to inject into the circulation approximately 7 grams of quinine and maintain that concentration for some time.

EFFECT OF BILE ON NORMAL, MALARIAL, AND BLACKWATER FEVER BLOOD CELLS

Subacute jaundice was regularly present in the individuals prior to the attack of hemoglobinuria. The physicians of Palestine consider the jaundice as an indication of danger and refuse to give quinine to such patients. This fact, together with the known hemolytic power of bile, suggested that this substance might play some causative rôle in hemoglobinuria. Tests were made, therefore, on the effect of ox bile, on blood cells of normal individuals as well as of those suffering with malaria or blackwater fever. The results showed that bile in a dilution of 1:25 caused prompt laking of cells of healthy or diseased individuals, while a dilution of 1:50 usually did not lake the blood cells. In either dilution the effect was practically the same on various types of blood.

EFFECT OF MIXTURE OF QUININE AND BILE

Experiments were now made to ascertain the associative action of bile and quinine in various proportions. Interesting results were obtained. Mixtures containing a 1:50 or 1:75 dilution of bile and 0.02 to 0.03 per cent of quinine invariably brought about laking, while the same concentrations of these substances acting separately had no effect. In other words concentrations of bile and quinine, separately incapable of producing laking, caused

marked hemolysis when acting in combination. These experiments were repeatedly made with human red cells both normal and malarial as well as with rabbit cells, always with the same results. The following are type experiments indicating the method of procedure, concentrations, and results.

Experiment 1. Ox bile was diluted with saline in the proportion of 1:9 and varying quantities added to a series of tubes containing the same amount of quinine. The volume in each case was made up to 1 cc., and 0.1 cc. of defibrinated blood added to each tube. The tubes were placed in the incubator for one hour and then observed at room temperature.

Quinine mgm.....	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.0
Bile (1:10), cc.....	0.1	0.2	0.3	0.4	0.5	0.6	0.0	0.3
Results.....	No hemolysis	Hemolysis in ½ hour	Immediate hemolysis				No hemolysis	No hemolysis

Experiment 2. Same procedure as experiment 1, except bile concentration constant and quinine varied.

Quinine mgm.....	0.4	0.2	0.1	0.05	0
Bile (1:25) cc.....	1.0	1.0	1.0	1.0	1
Results.....	Hemolysis in five minutes		Hemolysis in in ten minutes	Complete hemolysis one hour	Slight hemolysis one hour

Experiment 3. Effect of bile and quinine on blood of malaria patient. Blood taken at end of chill; serum yellowish. One-tenth cubic centimeter defibrinated blood added to each tube containing 1 cc. of bile-quinine mixture. Tubes incubated one hour at 37°C., then left at room temperature ten hours.

Bile (1:50), cc.....	1.0	1.0	1.0	1.0	0.0	0.0
Quinine mgm.....	0.2	0.3	0.4	0.0	2.0	0.0
Results, (per cent of cells laked)....	20	30	35	4.0	Trace	0.0

Experiment 4. Effect on blood of blackwater fever immediately after fresh attack of malaria. Blackwater fever January 23. Malaria March 1. Tests March 2. Procedure same as in experiment 3.

Bile (1:75), cc...	1.0	1.0	1.0	0.0	0.0	0.0
Quinine, mgm....	0.2	0.3	0.0	2.0	0.3	0.0
Malaria blood...	70 per cent hemolysis	Complete hemolysis	Trace	50 per cent hemolysis	No hemolysis	No hemolysis
Normal blood....	Trace	20 per cent hemolysis	Trace	No hemolysis	No hemolysis	No hemolysis

Experiment 5. Effect of quinine-bile mixture on normal blood. Procedure same as above.

BILE (1:50)	QUININE	BLOOD A		BLOOD B	
		Twenty-four hours	Forty-eight hours	Twenty-four hours	Forty-eight hours
cc.	*mgm.*				
1.0	0.2	±	+	±	+
1.0	0.3	+	+	±	+
1.0	0.4	+	+	+	++
1.0	0.0	−	Trace	−	Trace
0.0	0.4	−	−	−	−

Experiment 6. Effect of bile-quinine mixture on rabbit blood. Procedure as above.

					REMARKS
Bile (1:5).....	0.10	0.15	0.15	0.1	The volume was made up to 1 cc. Tubes left two hours at 37°C., then at room temperature
Quinine, mgm.....	0.2	0.2	0.0	0.0	
Results four hours....	−	+	−	−	
Results twenty-four hours.....	±	++	±	Trace	

Experiment 7. Effect of different kinds of biles. It became evident in the course of the experiments that different samples of bile varied in their effect. The following is a typical experiment. The procedure of the test is the same as above.

	BILE (1:50)	QUININE			REMARKS
		0.2 mgm.	0.3 mgm.	0 mgm.	
	cc.				
A (dark green).....	1	80 per cent hemolysis	Complete hemolysis	Trace	One hour at 37°C., then at room temperature
B (yellowish green)...	1	30 per cent hemolysis	50 per cent hemolysis	0.0	

The experiments cited above demonstrate hemolysis by combinations of bile and quinine solutions that are ineffective separately. Neither the bile salts nor the reaction of the bile seem to be the factors concerned. The tests were always made with solutions of neutral reaction, while the bile salts themselves did not give the effects of the whole bile.

EXPERIMENTAL HEMOGLOBINURIA

A series of attempts was made to produce hemoglobinuria in rabbits by the injection of bile and quinine. In some experiments varying doses of quinine were injected on a series of successive days, followed by a moderate dose of bile. In others the bile was injected first and then the quinine. In still others mixtures of the two substances were injected.

In one experiment only were definitely positive results obtained; in four others the results were doubtful and in the larger number of experiments the results were negative.

In the successful experiment a typical picture of hemoglobinuria was obtained.

Four cubic centimeter sterile ox bile was injected intravenously into a rabbit weighing 1100 grams. Five minutes later 30 mgm. of quinine hydrochloride was given by the same route. Before the injection, the serum was colorless. After the injection of the bile there was marked hemoglobinemia—such as can be obtained by laking 0.01 cc. of rabbit red cells in a fluid bulk—which diminished rapidly, so that two hours after the injection the serum was only slightly tinged with red. One hour after the injection of the quinine a small quantity of urine was obtained

which contained a fairly large amount of hemoglobin, during the next twenty-four hours only 8 cc. of urine being voided. This urine still contained hemoglobin but quinine was absent. The next day hemoglobin was absent but quinine was present.

The control animal receiving bile alone died immediately after the injection, while the one receiving quinine alone showed no effect whatever despite the proportionately large dose administered.

The doubtful results were all obtained by the injections of 40 mgm. of quinine on two succeeding days followed by 1 or 2 cc. of bile. In all the four experiments the urine collected during the twenty-four hours following the injection of bile contained traces of hemoglobin and albumin. The results were not, however, conclusive and could not be reobtained at will.

DISCUSSION

The series of observations reported in this paper seem to have a bearing on the mechanism of quinine hemoglobinuria. It appears that in the presence of bile quinine becomes highly hemolytic even in the small doses normally employed in the treatment of malaria. Some as yet undetermined constituent of bile has the power of rendering quinine hemolytic in one-tenth the concentration usually required. If we assume that the substance in question is liberated during the active destruction of blood cells in the course of repeated attacks of malaria, a simple theory of the mechanism of hemoglobinuria suggests itself. Hemoglobinuria patients invariably give a history of repeated attacks of malaria. In the course of these attacks cell products are constantly liberated in the circulation which may not at times be readily eliminated and thus reach a concentration higher than normal. At that time the injection of quinine causes hemolysis and hemoglobinemia which manifests itself by the appearance of hemoglobin in the urine.

Experiments on rabbits carried on thus far have not yielded satisfactory results because of the difficulty of reproducing the exact conditions developed by chance in human beings. Hemoglobinuria was produced by the injection of bile but the dose

injected bordered on lethality and consequently could not be relied on to give constant results. In four experiments mild hemoglobinuria was obtained by the injection of quinine followed by bile.

It is realized that the observations reported are of a fragmentary character. They are, however, of value in so far as they establish that quinine becomes actively hemolytic even in small doses when acting in combination with bile, a product of destruction of red blood cells.

The experiments are being continued with a view to finding some specific product of blood cell destruction which will increase the hemolytic power of quinine in the same manner as bile and the conditions required to produce hemoglobinuria experimentally in animals.

RESUMEN

La serie de observaciones relatadas en este artículo parecen tener cierta relación con el mecanismo de la hemoglobinuria de quinina. En presencia de bilis, la quinina se manifiesta altamente hemolítica aún en las pequeñas dósis comúnmente empleadas en el tratamiento de la malaria. Algún constituyente indeterminado de la bilis tiene el poder de hacer a la quinina hemolítica en una décima de la concentración usualmente requerida. Si asumimos que la substancia en cuestión se descarga durante la destrucción activa de las células de la sangre, después de repetidos ataques de malaria, esto en sí sugeriría una sencilla teoría del mecanismo de la hemoglobinuria. Los pacientes de hemoglobinuria invariablemente presentan una historia de repetidas recidivas maláricas. En el curso de estos ataques productos celulares son constantemente descargados en la circulación, que al no ser eliminados, alcanzan concentración abnormal. A veces la inyección de quinina causa hemólisis y hemoglobinuria que se manifiesta con la aparición de hemoglobina en la orina.

Los experimentos con conejos llevados a cabo no han dado resultados satisfactorios debido a la dificultad de reproducir las condiciones exactas que en oportunidades se desarrollan en el humano. Se produjo hemoglobinuria mediante la inyección de

bilis pero la dosis administrada se acercaba tanto a la letalidad que en consecuencia no se confía que dé resultados constantes. En cuatro experimentos se obtuvo hemoglobinuria benigna mediante inyección de quinina seguida de bilis.

Se comprende que las observaciones relatadas son de caracter fragmentario. Su valor consiste, sin embargo, en que establecen que la quinina se hace altamente hemolítica aun en pequeñas dosis en combinación con la bilis.

Se continúan estos experimentos con el propósito de averiguar si existe algun producto específico en la destrucción de las células rojas que aumente el poder hemolítico de la quinina, de igual manera que la bilis, y bajo las condiciones requeridas para producir la hemoglobinuria experimentalmente en animales.

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